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ANTIGENIC SPECIFICITY OF CLOSTRIDIUM BOTULINUM TYPES C, D, AND E

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The presently known five types of causative agents of botulism Cl. botulinum A, B, C, D, and E -- produce soluble toxins which have a similar physiological effect on the animal organism, but differ in their antigenic properties.

The antigenic specificity of botulin toxins of the A, B, and E types, according to the data of Chertkova et. al. (1948), and also Prevot and Brygoo (1953) is very high. Toxins of these types are neutralized only by homologous antitoxin serum. The specificity of toxins of C and D type is somewhat limited. Mason and Robison (1935), Prevot and Brygoo (1953), Guillaumie et. al. (1955) indicated in their works that massive amounts of the antitoxic sera C and D can neutralize in an overlapping fashion small doses of heterologous toxins, for which the neutralizing strength of the sera depends on the strain used in preparation of the toxin. Accordingly, the question of the validity of isolating the toxins C and D into separate types of Cl. botulinum antigens has arisen.

We set ourselves the goal to discover the extent of antigenic similarity of Cl. botulinum of types C, D, and E not only from the reaction of neutralization of their toxins, but also in experiments of overlapping passive and active immunization of animals and the precipitation reaction with bacterial antigens.

In experiments on neutralization of toxins and passive immunization use was made of rabbit antitoxin sera of the C and D types containing 100-200 AE per ml, and equine antitoxin serum of type E with an activity of 3000 AE per ml. Sera of the animals before immunization did not contain normal anti-

TABLE 1

Results of Testing Specificity of Botulin Toxins of the C, D, and E Types in Neutralization Experiments with White Mice

(a) Сыворотка		(d) Токсин		(f) Результат	(a) Сыворотка		(d) Токсин		(f) Результат
(b) тип	(c) количество (в AE)	(b) тип	(e) количество (в Dlm)		(b) тип	(c) количество (в AE)	(b) тип	(e) количество (в Dlm)	
C	1	C	250	2/0	D	1	D	1 000	4/0
	1	D	1	1/3		1	C	1	0/4
	100	D	50	4/0		50	C	50	2/0
	100	D	100	0/2		50	C	100	0/2
E	1	E	100	2/0		50	E	1	1/4
	1	D	1	0/2		50	E	2	0/5
	100	D	2	5/0		50	E	4	0/5
	100	D	4	0/5					

Remark. In tables 1-3 the numerator denotes the number of surviving animals, the denominator -- the number of animals succumbing.

LEGEND: a) Serum; b) type; c) number (in AE); d) Toxin, e) quantity (in Dlm); f) Result

bodies to toxins of the A, B, C, D, and E types.

Dry toxins of the C, D, and D types were prepared by salting out with ammonium sulfate from seven-day Cl. botulinum cultures of the strains C-91, D-359, and E-188-20. The activity of dry toxins of the C, D, and E types was, respectively, 30,000, 2000, and 400 Dlm per mg for white mice.

To discover neutralizing capacity specific amounts (in AE) of antibotulin sera of the C, D, and E types were mixed with different amounts (in Dlm) of homo- and heterologous toxins. After being kept for one hour at room temperature the mixture was introduced intravenously to white mice. In parallel, the value of the toxin Dlm was controlled in each experiment. The results can be estimated from the death of mice in the typical clinical pattern of botulism during the course of four days following administration of the mixture.

Table 1 data points to the presence of cross-neutralization reaction between sera and toxins of the C, D, and E types

TABLE 3

Determination of Extent of Antigenic Kinship of Botulin Toxins of the C, D, and E Types in Experiments on the Active Immunization of White Mice Using Sorbed Anatoxins

a Анатоксин		Напряженность иммунитета через 14—16 суток			
b тип	доза в ЕС (в 1 АЕ)	e тип токсина	f доза токсина (в Dcl)	g число мышей	h LD_{50} (в Dcl)
C	40	C	1 5 25	18/1 19/1 23/4	>25
		D	1 2 5	8/11 0/18 0/19	~1
D	20	D	10 30 100	10/5 8/7 2/9	28±9
		C	1 2 4	6/4 2/19 0/10	1,2±0,2
		E	0,5 1 2	5/2 1/6 0/5	<1
E	10	E	10 50	6/2 2/6	25±8
		D	0,5 1 2	7/0 5/3 1/6	1,2±0,2

LEGEND: a) Anatoxin; b) type; c) dose in EC (per 1 AE); d) Intensity of immunity in 14-16 days; e) tvpe of toxin; f) dose of toxin (in Dcl); g) number of mice; h) LD_{50} (in Dcl)

From the experimental data it is clear that the extent of antigenic similarity between botulin toxins of the C, D, and E types is very slight. Cross-neutralization of small doses (2-50 Dlm) of toxins was observed only when high (50-100 AE) concentrations of heterologous serum was used.

In order to more fully represent the antigenic specificity of types C, D, and E of Cl. botulinum, an investigation of

TABLE 2

Determination of the Extent of Antigenic Similarity of Botulin Toxins of the C, D, and E Types in Experiments on Passive Immunization of White Mice

Сыворотка (a)		Токсин (d)		(f)	(g)	Сыворотка (a)		Токсин (d)		(f)	(g)
(b) тип	(c) количество (в AE)	(b) тип	(e) количество (в Dlm)	Число мышей	LD_{50} (в Dlm)	(b) тип	(c) количество (в AE)	(b) тип	(e) количество (в Dlm)	Число мышей	LD_{50} (в Dlm)
C	100	C	2000	10/0	>2000	D	100	D	1 000	16/0	>1000
	100	D	10	10/0			100	C	20	8/2	
	100	D	30	9/0	45±10		100	C	60	5/5	56±15
	100	D	100	0/10			100	C	200	1/9	
E	100	E	250	8,0	>250	D	50	E	1	0,7	<1
	100	D	1	8,0			50	E	5	0,7	
	100	D	5	0,8	2,3±						
	100	D	20	0,8	±0,3						

LEGEND: a) Serum, b) type; c) number (in AE); d) Toxin; e) number (in Dlm); f) Number of mice; g) LD_{50} (in Dml)

in this case when the reaction was carried out at high serum doses (50-100 AE) and with small amounts of toxin (2-50 Dlm).

The antigenic similarity of botulin toxins of the C, D, and E types was manifest also in experiments on passive immunization. One hour after intravenous injection of antitoxin serum of the C, D, or E types to white mice, various doses of homo- and heterologous toxins were introduced intraparenterally. From the experimental results (treated according to the probit [7] method with modifications of Weiss, 1948), presented in Table 2, it follows that 100 AE of serum produced in white mice high immunity to the same toxin ($LD_{50} > 1000$ and > 2000) and an immunity many times lower to the toxin of another type ($LD_{50} \sim 50$ Dlm).

In order to explain the existence of cross immunity in actively immunized animals a single subcutaneous immunization of white mice using concentrated aluminum oxide hydrate-sorbed botulinic anatoxins of the C, D, and E types. In 16 days following immunization intensity of immunity to the toxins of these types was tested. The data presented in Table 3 shows that active immunization induced in the animals only slight immunity ($LD_{50} \sim 1$ Dcl for white mice) to heterologous toxin.

TABLE 4

Serological Relationships of Cl. botulinum Strains of the C, D, and P types According to the Precipitation Reaction

(a) Сыворотка			(e) Титр реакции преципитации с антигенами из микробных клеток Cl. botulinum разных типов															
			D			C							E					
(b) тип	(c) № штамма	(d) серологическая группа	359	7	185	91	185	121	6/п	185	186/3	468	2	6/п	188-20	513	115-35	110-35
	359	I	1:32	1:16	1:16	—	—	—	—	—	—	1:16	—	—	—	1:16	1:16	1:16
D	7	I	1:16	1:16	1:32		—	—	—	—	—	1:4	—	—	—	1:4	1:4	1:4
	165	I	1:16	1:16	1:16	—	—	—	-	—	—	1:4	—	—	—	1:4	1:4	1:4
C	91	I	—	—	—	1:32	1:32	1:32	1:16	1:16	1:16	—	—	—	—	—	—	—
	468	II	1:16	1:16	1:16					—	—	1:32	—	—	—	1:16	1:16	1:8
	188-20	I	—	—	—	—	—	—	—	—	—	—	1:16	1:32	1:32	—	—	
	513	II	1:8	1:8	1:8	—	—	—	—	—	—	1:8	—	—	—	1:16	1:8	1:8
	110-35	II	1:8	1:8	1:8	—	—	—	—	—	—	1:8	—	—	—	1:8	1:8	1:8

LEGEND: a) serum; b) type; c) number of strain; d) serological group; e) titer of precipitation reaction with antigen from Cl. botulinun bacterial cells of various types.

serological ties between strains of these types was undertaken. The 16 strains of the listed types available to us were tested in cross precipitation reactions with precipitating sera to 8 strains of Cl. botulinum C, D, and E. Serving as antigens for the reaction were boiled extract from suspensions (10 billion per ml) bacterial cells of 24-hour cultures of the corresponding strains. The precipitation reaction was conducted by layering onto undiluted serum -- the successive twofold dilutions of the antigen. The reaction results were recorded in 15 minutes. It follows from the data presented in Table 4 that the strains of type D, including also, the nontoxigenic -- No 165 and 7 -- represent one serological group i.e., these strains have a bacterial agent in common. Strains of each of the types C and E were distributed into two serological groups (I and II). Sera of type D did not precipitate strains in the first serological groups of types C and E, but reacted with the strains C-468, and E-513, 115-35, and 110-35, belonging to the serological group II of the appropriate type. Thus, by means of the precipitation reaction a bacterial antigen similar to the antigen present in the strains of the serological group II of the types C and E.

The above results on neutralization of toxins, passive and active immunization, the reaction of complement-fixation point to the antigenic independence of types C, D, and E of Cl. botulinum, which however have a certain mutual relationship. The extent of this relationship within the limits of the strains studied (D 3, C 7, and E-6) is very slight and cannot serve as the basis for merging types C and D Cl. botulinum into one type.

Conclusions

1. Botulin toxins of the C, D, and E types have some certain relationship; large doses of typical antitoxin sera are capable of neutralizing small amounts of heterogeneous toxin.

2. Active or passive immunization of animals against botulin toxins C, D, or E is accompanied by the development of very low resistance of animals to the toxins of the other group.

3. The strains Cl. botulinum D have a bacterial antigen (precipitinogen), inherent to certain strains of the types C and E.

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